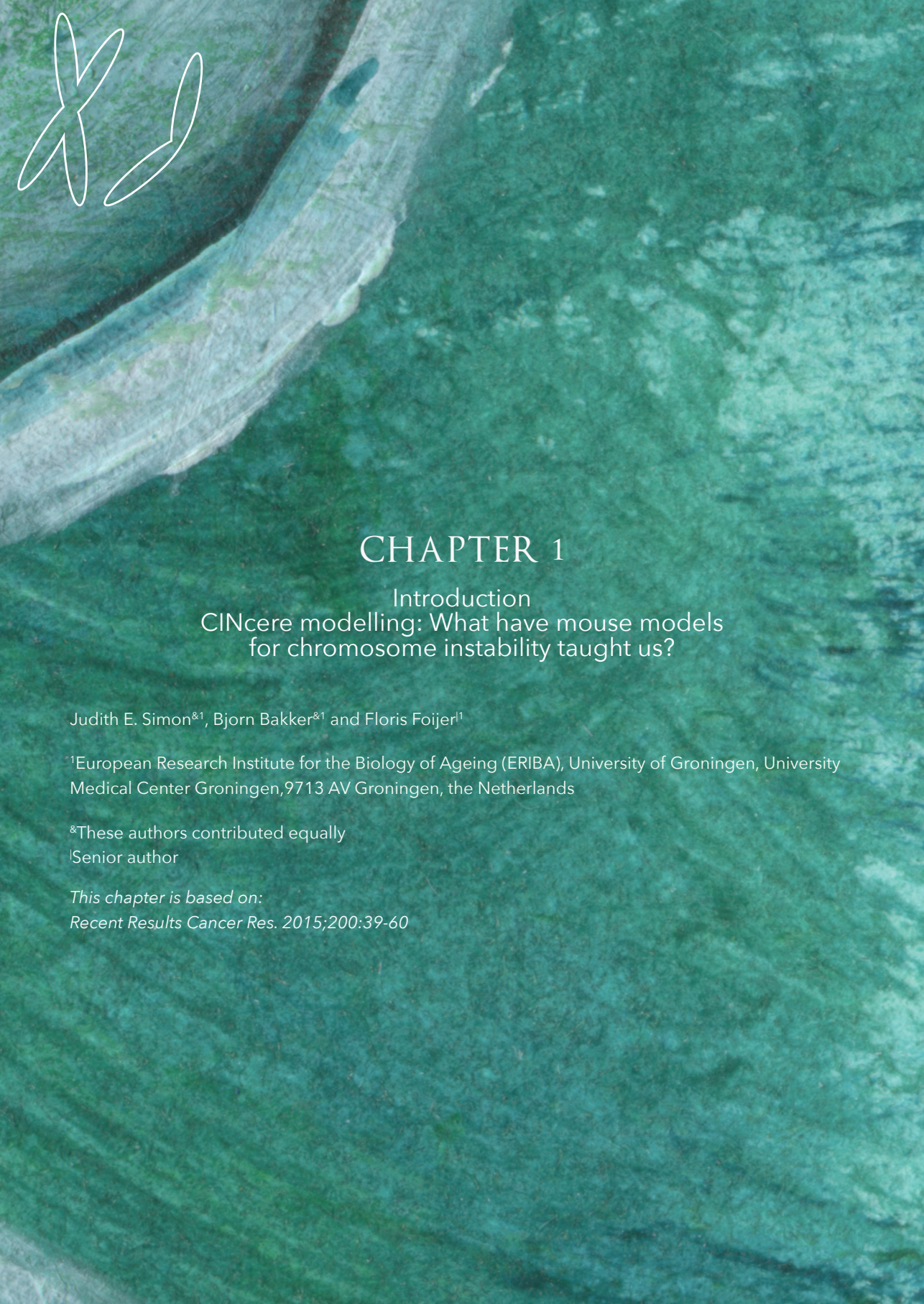

CHAPTER 1

Introduction
CINcere modelling: What have mouse models for chromosome instability taught us?

Judith E. Simon[&1], Bjorn Bakker[&1] and Floris Foijer[I1]

[1]European Research Institute for the Biology of Ageing (ERIBA), University of Groningen, University Medical Center Groningen,9713 AV Groningen, the Netherlands

[&]These authors contributed equally
[I]Senior author

This chapter is based on:
Recent Results Cancer Res. 2015;200:39-60

ABSTRACT

Chromosome instability (CIN) is a process leading to errors in chromosome segregation and results in aneuploidy, a state in which cells have an abnormal number of chromosomes. CIN is a hallmark of cancer, and furthermore linked to ageing and age-related diseases such as Alzheimer's. Various mouse models have been developed that model CIN to study the relationship between CIN, ageing and cancer. While these models reveal only a modest contribution of CIN to the initiation of cancer, they also clearly show that CIN is a powerful accelerator of cancer in a predisposed background. Other than cancer, CIN also appears to provoke premature ageing in some of the CIN models. In this review, we discuss the phenotypes of the various available mouse models, what we have learnt so far, and importantly, also which questions still need addressing.

INTRODUCTION

Chromosome instability and aneuploidy

During each cell division our genetic code is replicated, followed by the symmetrical segregation of all chromosomes into the emerging daughter cells. Cancer cells occasionally mis-segregate their chromosomes, a process known as chromosome instability (CIN), leading to cells with abnormal numbers of chromosomes, a state defined as aneuploid. In addition to whole chromosome abnormalities, CIN can also lead to structural abnormalities such as amplifications, deletions or translocations, either through defects in the DNA damage machinery or as a direct result of chromosome mis-segregation events[1]. Although numerical and structural abnormalities frequently coincide, in this review we will focus on how mouse models have contributed to our understanding of the consequences of whole chromosome instability.

David von Hansemann was the first to report abnormal chromosome numbers in carcinoma samples in 1890, long before the relationship between chromosomes and the genetic code had been established. Early in the 20th century, Theodor Boveri showed that aneuploidy leads to abnormal development or even death by injecting two sperm instead of one into sea urchin embryos. These observations led to the hypothesis that aneuploidy can lead to cancer or developmental defects[2–4]. Since then, many studies confirmed that CIN is a hallmark of human malignancies, affecting 2 out of 3 cancers[5]. More recently, aneuploidy has also been associated with ageing and age-related diseases[6]. For instance, trisomy for chromosome 21 is frequently found in plaques in Alzheimer patients' brains[7]. Conversely, people with Down syndrome develop early onset Alzheimer's disease[8], further emphasizing the relationship between trisomy 21 and neurodegenerative disease.

Although CIN has been associated with cancer for more than a century, we are only beginning to understand the consequences of CIN and aneuploidy at the cellular and molecular levels. CIN is believed to accelerate the evolution of cancer cells by facilitating gain of oncogenes and loss of tumour suppressor genes. Paradoxically, when modelled in yeast strains[9] or mouse embryonic fibroblasts (MEFs)[10], aneuploidy appears to decrease rather than enhance cell proliferation, suggesting that cancer cells find ways to cope with the adverse effects of aneuploidy. However, as transformation of aneuploid cells into aneuploid cancer cells can only occur *in vivo* by definition, animal models for CIN are essential to solve this paradox.

Provoking CIN in vivo

Several processes that safeguard correct chromosome segregation have been targeted to engineer mouse models for CIN. Figure 1 shows a schematic overview of a large number of genes that have been targeted for this purpose. One of the first models specifically designed to study the *in vivo* consequences of CIN is the Mad2 knockout mouse, targeting the spindle assembly checkpoint (SAC)[11]. The SAC prevents mis-segregation of chromosomes by inhibiting metaphase to anaphase progression until all chromosomes are properly attached to kinetochores in a bi-oriented fashion. Defects of the SAC therefore result in flawed chromosome segregation, which makes the SAC an attractive target to model CIN *in vivo*. A second means to induce CIN *in vivo* is by interfering with kinetochore integrity, a protein structure that connects the centromeric DNA to the mitotic spindle. This has been done by removing structural components of the kinetochore (*e.g.* CenpB, CenpC) or alternatively by stabilizing kinetochore-microtubule attachments through *e.g.* overexpressing Mad2 or Hec1[12,13]. Centrosomes are the microtubule-organizing centres in the cell from which the mitotic spindle emanates[14,15]. Abnormal centrosome numbers can either result in multipolar divisions or, when supranumeral centrosomes cluster, predispose for lagging chromosomes in mitosis[16]. Therefore, a third way to provoke CIN *in vivo* is by inducing centrosome amplification, *e.g.* through overexpression of Plk4[17,18]. A fourth approach to induce CIN *in vivo* is by disrupting the cohesion complex, a ring-like structure that holds the sister chromatids together during interphase. Cohesion defects have been modelled by abrogating components of the cohesion complex (e.g. SA1), but also by deregulating upstream players such as pRb[19–21]. Similarly, various other genes have been knocked out in the mouse that indirectly affect chromosome segregation.

In vivo consequences of CIN

In the last two decades, a large number of mouse models for chromosome instability have been engineered. Hereunder, we summarize the findings from these models asking the following questions:

1) Is CIN a *bona fide* instigator of cancer?

2) Which genes collaborate with CIN *in vivo* to convert aneuploid cells into aneuploid cancer cells?

3) What are the other consequences of CIN *in vivo*?

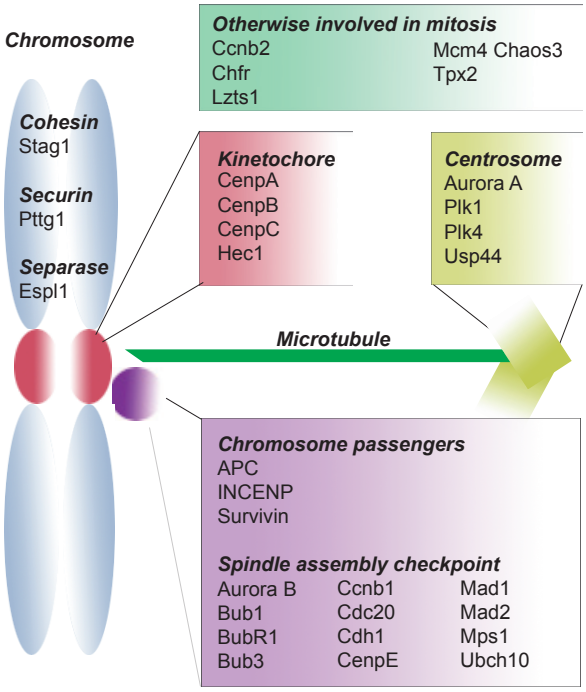

Figure 1 Schematic overview of various genes targeted to provoke CIN *in vivo*.

Can CIN initiate cancer?

CIN has detrimental consequences for cells grown *in vitro*[10,22,23], yet, two out of three human tumours are aneuploid[5,24]. This raises the question whether CIN is an initiating factor in cancer, a facilitator or merely a side effect of tumourigenesis. In the vast majority of all CIN-inducing mouse models (see Figure 1), full inactivation of the targeted genes resulted in early embryonic lethality. Although the time of embryonic death varied between genotypes (Table 1), embryos were typically lost before embryonic day 10, likely the result of aneuploidy in the inner cell mass of the developing embryos[11,24–27]. To circumvent embryonic lethality, phenotypes of heterozygous mice were monitored, or in some cases conditional alleles were engineered. Even though tumour phenotypes have been reported for many of these models (Table1), tumour incidence is relatively low, with fewer than 50% of the mice developing cancer. Moreover, tumours only arise late in the life of the mice, with latencies typically ranging from 12 to 24 months Table 1. The most frequent pathologies observed include lymphoma, lung and liver tumours. Furthermore, not all models develop spontaneous tumours, such as in case of the Bub family members (Bub1, Bub3, Rae1[28–33]). There is no clear correlation between the severity of the tumour

phenotypes and the mechanism that drove CIN in the mice (*i.e.* SAC mutation, cohesion defects, centrosome abnormalities etc.). Expression levels of the CIN-provoking genes, on the other hand, appear to be a better predictor of tumour incidence: phenotypes were most severe in cases where CIN-driving proteins were overexpressed to high levels (*e.g.* Mad2, Cyclin B1, Cyclin B2, Hec1, Plk4[13,17,34,35]) possibly because the relative effect on protein expression (several folds overexpression) was more dramatic than in heterozygous mice, where protein levels were typically reduced by ~50%. However, tumour latency is high in these models as well, suggesting that additional mutations must be required for aneuploid cells to become malignant.

Does CIN predispose cells to cancer?

Exposure to carcinogens is a powerful tool to assess tumour predisposition *in vivo*. Given the relatively weak tumour phenotypes of the mouse models described above, various CIN models were exposed to carcinogens (Table 1) to assess whether CIN is a powerful collaborator in transforming cells. Indeed, carcinogens aggravated the tumour phenotypes of some of the CIN mice, more than their control counterparts. For instance, when Mad1 heterozygous mice were treated with vincristine (a microtubule-depolymerizing agent) 40% of the mice developed, mostly lung tumours, while no tumours were detected in control-treated mice[36]. Likewise, carcinogens (NMBA or DMBA) accelerated tumourigenesis in Lzts1-deficient and Chfr-deficient mice, relative to control mice[37,38]. Furthermore, even in CIN mice without a tumour phenotype (*e.g.* $Bub1^{+/-}$, $Bub3^{+/-}$, $Rae1^{+/-}$ and $Bub3^{+/-}$ $Rae1^{+/-}$), DMBA treatment had a stronger tumour promoting effect in these CIN mice than on wildtype mice[31,32]. As carcinogens reduce tumour latency and increase tumour incidence in a CIN background, these experiments also indicate that additional mutations are required for a CIN cell to transform into a malignant cell.

Which genes collaborate with CIN in cancer?

To test which genetic alterations collaborate with CIN in tumourigenesis, various CIN models were crossed into cancer-predisposed backgrounds. For example, when CIN was combined with p53 heterozygosity, (Bub1, Espl1, Mps1[33,39,40]) tumour incidence dramatically increased while tumour latencies decreased. In all reported cases, tumours had lost the remaining p53 wild type allele, indicating that full p53 loss and CIN synergize in tumourigenesis[33,46]. However, since CIN also further increased tumour incidence of $p53^{null}$ mice, CIN must have facilitated cancer formation through additional genomic alterations as well. Additionally, CIN provoked by Bub1 hypomorphic alleles or Cyclin

B1 overexpression have been shown to accelerate tumours in a Apc^{min} background[33,35]. However, in other tumour predisposed backgrounds (*e.g.* pRb or Pten heterozygosity) CIN has no effect on tumour incidence[33].

CIN as a tumour suppressor

In some cases CIN can also act in a tumour suppressive manner. For instance, CIN driven by SA1 heterozygosity delays 3-methyl-colanthrene (3-MC)-induced fibrosarcoma and diethyl-nitrosamine (DEN)-induced liver tumours[41]. Similarly, while $Cdh1^{+/-}$ mice and $CenpE^{+/-}$ mice are more susceptible to spontaneous tumours, they are more resistant to carcinogenic insults than their wild type counterparts[42,43]. Furthermore, CIN can also delay tumourigenesis in some genetically predisposed models, for instance by delaying $p19^{Arf}$ or Pten loss-driven tumours[33,43]. Why then is CIN tumour promoting in one setting, but tumour suppressive in another? The answer might lie in the levels of CIN. CIN is quite toxic and provokes an 'aneuploidy stress' response in untransformed cells[9,10,23,44]. However, aneuploid cancer cells also exhibit this stress response[45,46], suggesting that aneuploid cancer cells still suffer from the disadvantageous effects of CIN. Therefore, the levels of CIN occurring in premalignant cells could be a determining factor for the outcome. The fact that $p19^{Arf}$ loss provokes aneuploidy itself fits with this hypothesis, as CenpE heterozygosity would exacerbate CIN to a level that is toxic for cancer cells[47]. However, further experiments are required to determine at what level CIN is beneficial for cancer cells and at what level the balance is tipped.

What other phenotypes are provoked by CIN?

There is increasing evidence that aneuploidy also occurs in untransformed tissues, with liver being the most well-known example. Up to half of both human and murine hepatocytes are aneuploid[48,49], but it is unclear why hepatocytes evolved to become aneuploid. One suggestion is that particular karyotypes are selected for during hepatotoxic insults, making the hepatocytes more resistant to injury[48]. Other studies quantified over 30% of normal human neuroblasts to be aneuploid[50,51], which has been suggested to contribute to the plasticity of neurons[52]. However, when provoked in a random fashion, CIN appears to mostly have disadvantageous effects on brain function, as mice heterozygous for Cdh1 exhibit defects in neuromuscular coordination and learning[42]. The interfollicular epidermal cells in mouse skin on the other hand appear to cope surprisingly well with CIN, as they tolerate full abrogation of the SAC provoked by Mad2 loss, which results in dramatic aneuploidy[44]. The hair follicle stem cells that reside in the same compartment do not cope at all and disappear, resulting in mice with functional skin but without hair[44].

Together these data clearly indicate that CIN is tolerated by some cell lineages but not others, underscoring the importance of *in vivo* modelling.

Linking ageing and CIN in vivo

Ageing is the time-dependent functional decline in the fitness of cells, organs and organisms. A common hallmark of ageing is genomic instability, as exemplified by genetic alterations in old blood cells[53,54]. Some of the CIN mouse models also suggest a role for aneuploidy in ageing. For instance, BubR1 hypomorphic mice are not only prone to severe aneuploidization, but also display progeroid pathologies. Similar to BubR1, combined Bub3/Rae1 haploinsufficiency also results in a premature ageing phenotype, albeit less severe than the BubR1 hypomorph[31], MEFs isolated from BubR1 hypomorphic mice express various ageing-associated markers such as p53, p21, $p19^{Arf}$ and $p16^{Ink4a}$. Interestingly, when $p16^{Ink4a}$ positive cells are killed *in vivo* using a $p16^{Ink4a}$-promotor regulated suicide construct, ageing pathologies induced by a reduction of BubR1 protein levels are dramatically delayed[55]. The pathologies observed in BubR1 hypomorphic mice mimic those of patients with multi-variegated aneuploidy (MVA), a disease that frequently coincides with mutations in *BUB1B*, the gene encoding BUBR1[56–58]. Furthermore, BubR1 expression levels decline with age providing further evidence for a role of BubR1 in ageing[55] of mice. Even more striking, when BubR1 is overexpressed, a dose-dependent delay in the onset of ageing is observed, as well as protection against developing chemically-induced tumours[59]. As discussed above, in most tested cases overexpression of CIN-controlling proteins increases CIN and predisposes for cancer[13,34,60]. Apparently BubR1 is the exception that forms the rule, but future work should reveal whether BubR1 has a unique role in the SAC or whether it has additional roles that can explain the beneficial effects of an overdose of BubR1.

What have we learnt from modelling CIN in the mouse so far?

As most tumours are aneuploid to some extent, CIN makes an attractive target for therapy. Therefore, understanding how CIN is signalled is crucial. A large number of mouse models have been engineered over the last 15 years specifically for this purpose, with a wide variety of phenotypes summarized in Table 1. Even though many of the targeted genes will have other roles in addition to safeguarding faithful chromosome segregation, some common conclusions can be drawn from the cumulative data. The first important conclusion is that CIN alone is not sufficient for efficient tumourigenesis and that CIN alone mostly has disadvantageous effects on cell proliferation. This has important implications for therapy targeting aneuploid cancer, as discussed below. A

second conclusion is that CIN facilitates tumourigenesis efficiently in some tumour-predisposed backgrounds, chemical or genetic. However, when CIN is aggravated and becomes too severe, it can actually suppress tumour formation in the mouse, which can also be exploited in cancer therapy. A third and perhaps the most important conclusion is that several unaddressed questions remain before we can develop therapeutic strategies targeting aneuploid cell progeny, some of which are discussed below. Although all models discussed here were designed to study the consequences of CIN *in vivo*, the majority mimic a situation that is not typically found in human cancers, as loss of genes that regulate chromosome segregations are rarely lost in human cancer[26,61]. Even though these models mimic chromosome mis-segregation and its consequences, overexpression of CIN-modulating genes is more common (*e.g.* Mad2 overexpression, which is seen in many tumours[34,62]). Possibly, mimicking the CIN-provoking mutations that are found in human cancers would result in a physiologically more relevant CIN level, thus adding to our understanding of CIN and its role in tumourigenesis.

Questions that need addressing

Which mutations make an aneuploid cell an aneuploid cancer cell?

Some tumour suppressor genes, (*e.g.* p53) were found to accelerate the malignant transformation of aneuploid cells, but the mechanism behind this collaboration remains unclear. As CIN alone is a poor initiator of cancer, pathways that convert aneuploid cells to aneuploid cancer cells will be important therapeutic targets. So far, CIN-collaborating genes were picked in an 'educated guess' approach. However, to identify in an unbiased fashion the pathways that convert CIN cells into CIN cancer cells, (*in vivo*) genetic screens are required.

At what rate is CIN tumourigenic and at what levels tumour suppressive?

The effects of CIN across the various mouse models are diverse, but it is unclear why. It is inevitable that the levels of CIN are different among the various CIN models, but there is no clear correlation between the levels of aneuploidy and the resulting phenotype based on the available data. However, as the level of CIN might determine whether tumours are promoted or are suppressed[47], high resolution quantification of CIN will be crucial when targeted in therapy. Furthermore, even though aneuploidy is a hallmark of cancer, the actual rates of chromosome mis-segregation (*i.e.* the CIN rates) in human cancer are unknown. To quantify these, primary (tumour) cells need to be fully karyotyped at the single cell level at various stages. So far, most studies have relied on metaphase-spread based (spectral) karyotyping using dividing cell populations, such as primary

MEFs or tumour cell lines. However, this technique cannot be applied to most primary tumour cells as they do not divide frequently[63,64]. A new, but costly approach to quantify karyotypes of single cells is next-generation sequencing (NGS). However, to quantify aneuploidy full coverage (or even multiple coverage) per cell is not a requirement. 1-2% coverage per cell will be sufficient to quantify chromosome numbers for an individual cell, allowing sequencing libraries of many cells to be pooled in each sequencing lane. Single-cell karyotyping will allow us to faithfully measure *in vivo* mis-segregation rates (*i.e.* CIN) by assessing subtle karyotype differences between cells within one tumour (karyotype heterogeneity). Such technology will allow us to determine at which rate CIN is tumourigenic or tumour suppressive in mouse models and what the CIN rates are in human primary tumours.

What determines the tissue-specific response to CIN?

There is a marked difference as to how cell lineages respond to CIN. For instance, CIN is highly toxic to embryonic stem cells[65], but quite well tolerated by interfollicular epidermal cells[44], hepatocytes and possibly neurons[49–52]. As of yet, it remains unclear what determines this differential response. Possibly, some cell lineages such as stem cells, induce a stronger stress response upon aneuploidy, resulting in apoptosis or differentiation. Alternatively, aneuploidy-tolerating cells spend more time in pro-metaphase and therefore have more time to correct improper kinetochore-microtubule attachments, thus reducing the mis-segregation rates and therefore reducing aneuploidy to tolerable levels. Indeed some cell types tolerate at least some aneuploidy including neurons and hepatocytes. However, further *in vivo* experiments are required to assess which molecular pathways make up the response to aneuploidy at the tissue level and how the differential wiring of these pathways in different cell lineages determines the fate of aneuploid cells.

What are the molecular mechanisms that explain the link between CIN and ageing?

Some of the CIN mouse models exhibit a premature ageing phenotype, most clearly knockout models of Bub family proteins (BubR1, Bub3/Rae1)[31,66]. Conversely, BubR1 transgenic mice show increased lifespan, clearly implicating BubR1 with ageing[59]. This data, together with the observation that BubR1 expression decreases with ageing in wild type animals[31,66], suggest that CIN may play a role in natural ageing. Why were phenotypes only described for Bub protein members? Possibly, (subtle) signs of premature ageing were overlooked in other CIN models, as these models were developed specifically to study the relationship between CIN and cancer and not ageing[67]. Indeed, a more detailed analysis of transcriptomes of Mad2^{null} epidermal cells suggests an ageing-like response

in murine skin following SAC abrogation[44], suggesting that CIN indeed provokes a premature ageing response in untransformed tissue. However, more detailed and high resolution mapping of CIN in ageing human tissues is required to confirm physiological relevance for a potential link between CIN and ageing. When this link is confirmed, the underlying molecular mechanisms that link CIN and ageing should be elucidated, employing exciting and possibly new, more human relevant CIN mouse models.

What is the potential of CIN-targeting therapy?

Aneuploidy is a hallmark of cancer and selectively killing aneuploid cells would therefore be a powerful means to treat cancer. The various mouse models for CIN have revealed that there are three possible outcomes for aneuploid cell progeny depending on the tissue affected: 1) cell death (*e.g.* in case of hair follicle stem cells), 2) cellular senescence (evidenced by premature ageing and upregulation of the senescence marker $p16^{Ink4a}$ and 3) tolerance of aneuploidy (Figure 2). The latter outcome is the most dangerous, as proliferating aneuploid cells can further evolve into aneuploid cancer cells. Therefore, to target aneuploid cancer, those cells that tolerate aneuploidy will need to be forced to either commit suicide or become senescent. There are multiple ways as to how such therapy could work, ranging from broad-spectrum to highly 'personalized' therapies. As discussed above, too much CIN is detrimental to cells[47]. Therefore, further increasing CIN in aneuploid tumours could be a broad-spectrum way to target aneuploid cancer cells. Indeed, mild CIN renders cells more sensitive to therapeutics that exacerbate CIN such as low doses taxol[68]. However, the inherent risk to this therapy is that untransformed (non-CIN) cells will also be exposed to CIN and might convert into a new CIN tumour over time. A second approach of targeting CIN cells is by modulating the pathways that regulate cell fate following aneuploidization. In this approach, the pathways that result in cell death of (embryonic) stem cells following CIN are artificially activated in aneuploid cancer cells, resulting in cancer cell death. However, before feasibility of such therapy can be assessed, CIN-responsive pathways need to be mapped first. Instead of targeting aneuploidy-signalling pathways, therapy can also target the downstream consequences of CIN. For instance, one common response to aneuploidy is a deregulation of cellular metabolism, which affects untransformed cells as well as cancer cells[10,22,46]. The first proof of principle evidence for such therapy is just emerging. A recent study shows that the energy stress inducer AICAR and the Hsp90 inhibitor 17-AAG can selectively kill aneuploid (cancer) cells by enhancing aneuploidy-induced stress[69]. The next step to this will be to test whether this is also effective *in vivo*. A fourth 'personalised medicine' approach to tackle aneuploid cancer is by targeting the mutation that is driving CIN.

One candidate for such therapy is Hec1, as it is frequently overexpressed in a variety of cancers. Indeed, inhibition of the Hec1/Nek2 pathway results in reduced tumour growth in a xenograft mouse model[70], providing proof of principle evidence for this approach. Similarly, gene products that collaborate with CIN in transformation can be targeted using molecular therapy. For the latter, we first need to identify candidate targets, for instance in *in vivo* genetic screens. However, for molecular therapy full sequencing of the tumour is a requirement. As sequencing costs are rapidly decreasing and the number of specific pathway inhibitors are rapidly increasing, this approach might become feasible in the near future.

ACKNOWLEDGEMENTS

We thank Georges Janssens and Klaske Schukken for critically reading this manuscript and fruitful discussion. This work was supported by Stichting Kinderoncologie Groningen (SKOG) funding and Dutch Cancer Society grant RUG 2012-5549.

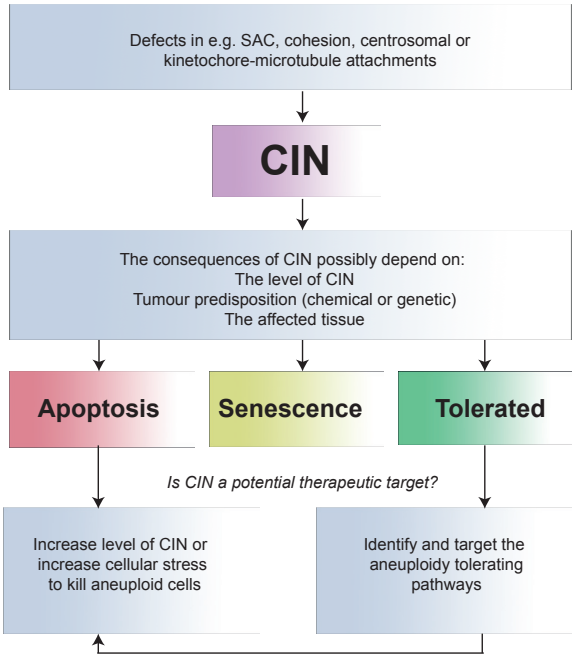

Figure 2 Flowchart summarizing the *in vivo* consequences of CIN and therapeutic promise.

Table 1 List of various mouse models engineered to provoke CIN *in vivo*, with phenotypes and observed aneuploidy levels *in vivo* and *in vitro* where quantified.

Group	Gene	-/-	+/-	Cancer predisposed (chemical or genetic collaboration)
SPINDLE ASSEMBLY CHECKPOINT	AuroraB	EL	>60%; 24 mo	Tumour suppression upon DMBA+T-PA-induced (not sig.)
	Bub1	EL (E6.5)	VNODD	DMBA-induced - 57%, $p53^{+/-}$; 16.6 mo
	Bub1 hypomorph	n/a	50%; 12 mo	78%, $p53^{+/-}$; 12 mo
	Bub3	EL (E6.5)	VNODD	DMBA-induced
	Bub3; Rae1	ND	VNODD	DMBA-induced
	BubR1	EL (E6.5)	VNODD	DMBA-induced
	BubR1 hypomorph	n/a	Premature ageing	DMBA- & azoxymethane-induced
	BubR1 overexpression	n/a	Delayed ageing	DMBA-induced, but decreased susceptibility than WT
	Ccnb1 (Cyclin B1) overexpression	n/a	>75%; (lung, lymphoma, liver, lipoma)	~80%, APC+/min; 40% (WT comparable; skin), DMBA-treatment
	Cdc20 AAA mutant (does not bind to Mad2)	EL (E12.5)	50%; 24 mo	ND
	Cdh1	EL (E10.5)	17% females – (mammary); mild brain abnormalities and altered behaviour	Tumour suppression upon TPA/DMBA treatment
	CENPE	EL (<E7.5)	20% (lung, spleen); 19-21 mo	Tumour suppression upon DMBA treatment or $p19^{Arf}$ loss
	Mad1	EL	20% (lung); 18-20 mo	Vinicristine-induced
	Mad2	EL	30% (lung); 18 mo	ND
	Mad2 overexpression	n/a	50% (lymphomas, lung & liver); 20 mo	DMBA-induced
	Mps1 (T-cell restricted)	VVNOD	~50% (lymphoma) 17 mo	100%, $p53^{+/-}$; 5 mo
	Rae1	EL (E6.5)	No spont. tumourigenesis	DMBA-induced
	Ubch10 overexpression	n/a	Expression level dependent: 40-80% (lymphoma, lung adenoma, lipoma and liver and skin)	Yes, but not significantly different compared to wild type
KINETOCHORE	CENPA	EL (E6.5)	VNODD	ND
	CENPB	VNODD	VNODD	ND
	CENPC	EL (E3.5)	VNODD	ND
	Hec1 overexpression	n/a	13% (lung), 26% (liver); 67 wk, 60 wk	ND

Aneuploidy in tissues	Aneuploidy in MEFs	Implicated in human cancer [26]	Reference
ND	ND		[60]
ND	ND		[32,33]
ND	15% (seg. defects)		[32,33]
10% (splenocytes)	20%		[28,29,31,71]
40% (splenocytes)	40%		[29,31]
Polyploidy in megakaryocytes	15%	Yes	[30,66]
30% (splenocytes)	35%	Yes (MVA)	[66]
1% (splenocytes)	9% (WT comparable)		[59]
hi 20%, lo 12% (splenocytes)	31% (ctrl. 15%)		(Nam and van Deursen, 2014)
35% ($Cdc20^{AAA/+}$, splenocytes)	28% ($Cdc20^{AAA/+}$ and 52% of $Cdc20^{AAA/AAA}$)		[72]
ND	Increased (not quantified)		[42]
40% (splenocytes)	20% (up to 70% at high passage)		[43,73]
ND	10%		[36]
ND	55%		[11,74]
Aneuploid tumours (not quantified)	50%	Yes	[34]
>90% of cells aneuploid	ND		(Foijer et al., 2014)
10% (splenocytes)	20%		[29,31]
4-19% hi-lo, 5 mo (splenocytes); 52-64% (lymphoma)	28-33% (WT 13%)	Yes	(van Ree et al., 2010)
Chromosome missegregation in E6.5 $CENPA^{-/-}$ embryos	n/a		[75]
ND	ND		[76–78]
Aberrant mitosis and micronuclei in early embryos	n/a		[79]
ND	25%	Yes	[13]

Table 1 Continued.

Group	Gene	-/-	+/-	Cancer predisposed (chemical or genetic collaboration)
COHESION	Espl1 (separase)	EL (E6.5)	Espl1$^{+/H}$; VNODD	86% (lymphomas), p53$^{-/-}$; 4 mo – 50% (carcinoma), p53$^{+/-}$
	Espl1 overexpression (mammary restricted)	n/a	80% (mammary), 11 mo	100% (mammary), p53$^{+/-}$; 14 mo
	Stag1 (exon 3 and 4, encoding SA1-cohesin subunit)	EL (beween E12.5 to E18.5)	40-50% (haematoma, lung, fibrosarcoma, liver, vascular, pancreas); 24 mo	Resistance to 3MC and DEN induced fibrosarcomas and liver tumours
	Pttg (securin)	Reduced testis, spleen and thymus weight.	n/a	Tumour protective, pRb$^{+/-}$
	Pttg (securin) overexpression	n/a	Enlarged pituitary; altered nuclear morphology	>80% (pituitary), pRb$^{+/-}$; 10 mo
CHROMOSOME PASSENGERS	APC/MIN	EL (<E8.5)	Intestinal tumours; 3 mo	ND
	Incenp	EL (3.5-8.5)	VNODD	ND
	Survivin	EL (6.5)	VNODD	ND
CENTROSOME	Aurora A overexpression (mammary restricted)	n/a	Increased p16 expression	45%, p53$^{-/-}$ (mammary gland); 4.5 mo
	Plk1	EL (E10.5)	27.5% (lymphoma, lung); 12.5-17.5 mo	100% (lymphoma, lung), p53$^{-/-}$
	Plk4 overexpression (CNS restricted)	n/a	Microcephaly, 100% post-natal lethality; <1 wk	100% lethality, p53$^{-/-}$; 5 mo
	Usp44	VNODD	Usp44$^{+/-}$ 20%, Usp44$^{-/-}$ 50%; 15 mo (lung, liver, lymphoma, sarcoma)	n/a
OTHERWISE INVOLVED IN MITOSIS	Ccnb2 (Cyclin B2) overexpression	n/a	>70% (lung, lymphoma, liver, lipoma); 14 mo	>80%, APC$^{+/min}$; >80% (lung), DMBA-treatment
	Chfr	VNODD	Chfr-/- 50%; 20 mo	DMBA-induced
	Mcm4 Chaos3	Chaos3/- EL (E14.5)	Mcm4Chaos3/+ (mammary); 12 mo	ND
	Tpx2	EL (E8.5)	53% (lymphoma, lung)	no

EL = Embryonic lethal; VNODD = Viable, no overt developmental defects; ND = Not determined; n/a = Not applicable

Aneuploidy in tissues	Aneuploidy in MEFs	Implicated in human cancer [26]	Reference
57% (splenocytes); 84% (bone marrow)	ND	Yes	[40]
>80% (mammary tumours)	ND		[80]
40% (fetal liver)	>70%		[41]
ND	15% (WT 1%)		[81,82]
ND	ND		[83,84]
Aneuploidy and abnormal mitosis in crypt cells	Increased, not quantified		[85–88]
Abnormal nuclear morphology hyperdiploid content in E3.5 embryos	n/a		[89]
Giant nuclei in early embryos	n/a		[89]
ND	13.6%		[90,91]
12% (splenocytes)	ND		[92]
31.7% centrosome amplification (neural stem cells); >60% aneuploidy of chr. 18 in $p53^{-/-}$	ND		[18]
8%, 5 mo; 16%, 15%, 15 mo (splenocytes)	18% (WT 13%)	Yes	[93]
18% (splenocytes)	36% (ctrl. 16%)		(Nam and van Deursen, 2014)
ND	25%		(Yu et al., 2005)
ND	ND		(Shima et al., 2007)
18,3%, 16 wk; 27%, 90 wk (splenocytes) 48.9%, 90 wk (lymphomas)			(Aguirre-Portolés et al., 2012)

REFERENCES

1. Janssen, A., van der Burg, M., Szuhai, K., Kops, G. J. P. L. & Medema, R. H. Chromosome segregation errors as a cause of DNA damage and structural chromosome aberrations. *Science* **333,** 1895–8 (2011).
2. Bignold, L. P., Coghlan, B. L. D. & Jersmann, H. P. a. Hansemann, Boveri, chromosomes and the gametogenesis-related theories of tumours. *Cell Biol. Int.* **30,** 640–4 (2006).
3. Boveri, T. Concerning the origin of malignant tumours by Theodor Boveri. Translated and annotated by Henry Harris. *J. Cell Sci.* **121 Suppl,** 1–84 (2008).
4. Boveri, T. & Manchester, K. L. Theodor Boveri - - the origin of malignant tumours. *Trends Cell Biol.* **5,** 384–387 (1995).
5. Duijf, P. H. G., Schultz, N. & Benezra, R. Cancer cells preferentially lose small chromosomes. *Int. J. Cancer* (2012). doi:10.1002/ijc.27924
6. Faggioli, F., Wang, T., Vijg, J. & Montagna, C. Chromosome-specific accumulation of aneuploidy in the aging mouse brain. *Hum. Mol. Genet.* 1–8 (2012). doi:10.1093/hmg/dds375
7. Iourov, I. Y., Vorsanova, S. G., Liehr, T. & Yurov, Y. B. Aneuploidy in the normal, Alzheimer's disease and ataxia-telangiectasia brain: differential expression and pathological meaning. *Neurobiol. Dis.* **34,** 212–20 (2009).
8. Lai, F. & Williams, R. S. A prospective study of Alzheimer disease in Down syndrome. *Arch. Neurol.* **46,** 849–53 (1989).
9. Torres, E. M. *et al.* Effects of Aneuploidy on Cellular Physiology and Cell Division in Haploid Yeast. *Science (80-.).* **317,** 916–924 (2007).
10. Williams, B. R. *et al.* Aneuploidy Affects Proliferation and Spontaneous Immortalization in Mammalian Cells. *Science (80-.).* **322,** 703–709 (2008).
11. Dobles, M., Liberal, V., Scott, M. L., Benezra, R. & Sorger, P. K. Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein Mad2. *Cell* **101,** 635–645 (2000).
12. Kabeche, L. & Compton, D. a. Checkpoint-independent stabilization of kinetochore-microtubule attachments by Mad2 in human cells. *Curr. Biol.* **22,** 638–44 (2012).
13. Diaz-Rodríguez, E., Sotillo, R., Schvartzman, J.-M. & Benezra, R. Hec1 overexpression hyperactivates the mitotic checkpoint and induces tumour formation in vivo. *Proc. Natl. Acad. Sci. U. S. A.* **105,** 16719–24 (2008).
14. Ganem, N. J., Storchova, Z. & Pellman, D. Tetraploidy, aneuploidy and cancer. *Curr. Opin. Genet. Dev.* **17,** 157–62 (2007).
15. Gordon, D. J., Resio, B. & Pellman, D. Causes and consequences of aneuploidy in cancer. *Nat. Rev. Genet.* **13,** 189–203 (2012).
16. Ganem, N. J., Godinho, S. a & Pellman, D. A mechanism linking extra centrosomes to chromosomal instability. *Nature* **460,** 278–82 (2009).
17. Ko, M. a *et al.* Plk4 haploinsufficiency causes mitotic infidelity and carcinogenesis. *Nat. Genet.* **37,** 883–8 (2005).
18. Marthiens, V. *et al.* Centrosome amplification causes microcephaly. *Nat. Cell Biol.* **15,** 731–40 (2013).
19. van Harn, T. *et al.* Loss of Rb proteins causes genomic instability in the absence of mitogenic signaling. *Genes Dev.* **24,** 1377–88 (2010).
20. Manning, A. L., Longworth, M. S. & Dyson, N. J. Loss of pRB causes centromere dysfunction and chromosomal instability. *Genes Dev.* **24,** 1364–76 (2010).
21. Coschi, C. H. *et al.* Mitotic chromosome condensation mediated by the retinoblastoma protein is tumour-suppressive. *Genes Dev.* **24,** 1351–63 (2010).
22. Torres, E. M., Williams, B. R. & Amon, A. Aneuploidy: cells losing their balance. *Genetics* **179,** 737–46 (2008).
23. Kops, G. J. P. L., Foltz, D. R. & Cleveland, D. W. Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint. *Proc. Natl. Acad. Sci. U. S. A.* **101,** 8699–704 (2004).
24. Weaver, B. A. A. & Cleveland, D. W. Does aneuploidy cause cancer? *Curr. Opin. Cell Biol.* **18,** 658–667 (2006).
25. Foijer, F., Draviam, V. M. & Sorger, P. K. Studying chromosome instability in the mouse. *Biochim. Biophys. Acta* **1786,** 73–82 (2008).

26. Schvartzman, J.-M., Sotillo, R. & Benezra, R. Mitotic chromosomal instability and cancer: mouse modelling of the human disease. *Nat. Rev. Cancer* **10,** 102–15 (2010).
27. Holland, A. J. & Cleveland, D. W. Boveri revisited: chromosomal instability, aneuploidy and tumourigenesis. *Nat. Rev. Mol. Cell Biol.* **10,** 478–487 (2009).
28. Kalitsis, P. Bub3 gene disruption in mice reveals essential mitotic spindle checkpoint function during early embryogenesis. *Genes Dev.* **14,** 2277–2282 (2000).
29. Babu, J. R. *et al.* Rae1 is an essential mitotic checkpoint regulator that cooperates with Bub3 to prevent chromosome missegregation. *J. Cell Biol.* **160,** 341–53 (2003).
30. Wang, Q. *et al.* BUBR1 deficiency results in abnormal megakaryopoiesis. *Blood* **103,** 1278–85 (2004).
31. Baker, D. J. *et al.* Early aging-associated phenotypes in Bub3/Rae1 haploinsufficient mice. *J. Cell Biol.* **172,** 529–40 (2006).
32. Jeganathan, K., Malureanu, L., Baker, D. J., Abraham, S. C. & van Deursen, J. M. Bub1 mediates cell death in response to chromosome missegregation and acts to suppress spontaneous tumourigenesis. *J. Cell Biol.* **179,** 255–67 (2007).
33. Baker, D. J., Jin, F., Jeganathan, K. B. & van Deursen, J. M. Whole chromosome instability caused by Bub1 insufficiency drives tumourigenesis through tumour suppressor gene loss of heterozygosity. *Cancer Cell* **16,** 475–86 (2009).
34. Sotillo, R. *et al.* Mad2 overexpression promotes aneuploidy and tumourigenesis in mice. *Cancer Cell* **11,** 9–23 (2007).
35. Baker, D. J., Jin, F. & van Deursen, J. M. The yin and yang of the Cdkn2a locus in senescence and aging. *Cell Cycle* **7,** 2795–802 (2008).
36. Iwanaga, Y. *et al.* Heterozygous deletion of mitotic arrest-deficient protein 1 (MAD1) increases the incidence of tumours in mice. *Cancer Res.* **67,** 160–6 (2007).
37. Yu, X. *et al.* Chfr is required for tumour suppression and Aurora A regulation. *Nat. Genet.* **37,** 401–6 (2005).
38. Vecchione, A., Croce, C. M. & Baldassarre, G. Fez1/Lzts1 a new mitotic regulator implicated in cancer development. *Cell Div.* **2,** 24 (2007).
39. Baker, D. J., Weaver, R. L. & van Deursen, J. M. p21 both attenuates and drives senescence and aging in BubR1 progeroid mice. *Cell Rep.* **3,** 1164–74 (2013).
40. Mukherjee, M. *et al.* Separase loss of function cooperates with the loss of p53 in the initiation and progression of T- and B-cell lymphoma, leukemia and aneuploidy in mice. *PLoS One* **6,** e22167 (2011).
41. Remeseiro, S. *et al.* Cohesin-SA1 deficiency drives aneuploidy and tumourigenesis in mice due to impaired replication of telomeres. *EMBO J.* **31,** 2076–89 (2012).
42. García-Higuera, I. *et al.* Genomic stability and tumour suppression by the APC/C cofactor Cdh1. *Nat. Cell Biol.* **10,** 802–11 (2008).
43. Weaver, B. a a, Silk, A. D., Montagna, C., Verdier-Pinard, P. & Cleveland, D. W. Aneuploidy acts both oncogenically and as a tumour suppressor. *Cancer Cell* **11,** 25–36 (2007).
44. Foijer, F. *et al.* Spindle checkpoint deficiency is tolerated by murine epidermal cells but not hair follicle stem cells. *Proc. Natl. Acad. Sci. U. S. A.* (2013). doi:10.1073/pnas.1217388110
45. Dürrbaum, M. *et al.* Unique features of the transcriptional response to model aneuploidy in human cells. *BMC Genomics* **15,** 139 (2014).
46. Foijer, F. *et al.* Chromosome instability induced by Mps1 and p53 mutation generates aggressive lymphomas exhibiting aneuploidy-induced stress. *Proc. Natl. Acad. Sci.* (2014). doi:10.1073/pnas.1400892111
47. Silk, A. D., Zasadil, L. M., Holland, A. J., Vitre, B. & Cleveland, D. W. Chromosome missegregation rate predicts whether aneuploidy will promote or suppress tumours. (2013). doi:10.1073/pnas.1317042110
48. Duncan, A. W. *et al.* Aneuploidy as a mechanism for stress-induced liver adaptation. *J. Clin. Invest.* **122,** 3307–3315 (2012).
49. Duncan, A. W. *et al.* Frequent aneuploidy among normal human hepatocytes. *Gastroenterology* **142,** 25–8 (2012).
50. Rehen, S. K. *et al.* Chromosomal variation in neurons of the developing and adult mammalian nervous system. *Proc. Natl. Acad. Sci. U. S. A.* **98,** 13361–6 (2001).
51. Rehen, S. K. *et al.* Constitutional Aneuploidy in the Normal Human Brain. **25,** 2176–2180 (2005).
52. Kingsbury, M. a *et al.* Aneuploid neurons are functionally active and integrated into brain circuitry. *Proc. Natl. Acad. Sci. U. S. A.* **102,** 6143–7 (2005).
53. Forsberg, L. a *et al.* Age-related somatic structural changes in the nuclear genome of human blood cells. *Am. J. Hum. Genet.* **90,** 217–28 (2012).

54. López-Otín, C., Blasco, M. a., Partridge, L., Serrano, M. & Kroemer, G. The Hallmarks of Aging. *Cell* **153,** 1194–1217 (2013).
55. Baker, D. J. *et al.* Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* **479,** 232–6 (2011).
56. Hanks, S. *et al.* Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in BUB1B. *Nat. Genet.* **36,** 1159–61 (2004).
57. Hanks, S. *et al.* Comparative genomic hybridization and BUB1B mutation analyses in childhood cancers associated with mosaic variegated aneuploidy syndrome. *Cancer Lett.* **239,** 234–8 (2006).
58. Matsuura, S. *et al.* Monoallelic BUB1B Mutations and Defective Mitotic-Spindle Checkpoint in Seven Families With Premature Chromatid Separation (PCS) Syndrome. **367,** 358–367 (2006).
59. Baker, D. J. *et al.* Increased expression of BubR1 protects against aneuploidy and cancer and extends healthy lifespan. *Nat. Cell Biol.* **15,** 96–102 (2013).
60. Fernández-Miranda, G. *et al.* Genetic disruption of aurora B uncovers an essential role for aurora C during early mammalian development. *Development* **138,** 2661–72 (2011).
61. Foijer, F. in *Aneuploidy in health and disease* (ed. Storchova, Z.) 59–78 (Intech, 2012).
62. Hernando, E. *et al.* Rb inactivation promotes genomic instability by uncoupling cell cycle progression from mitotic control. *Nature* **430,** 797–802 (2004).
63. Mitchison, T. J. The proliferation rate paradox in antimitotic chemotherapy. *Mol. Biol. Cell* **23,** 1–6 (2012).
64. McGranahan, N., Burrell, R. a, Endesfelder, D., Novelli, M. R. & Swanton, C. Cancer chromosomal instability: therapeutic and diagnostic challenges. *EMBO Rep.* **13,** 528–38 (2012).
65. Burds, A. a, Lutum, A. S. & Sorger, P. K. Generating chromosome instability through the simultaneous deletion of Mad2 and p53. *Proc. Natl. Acad. Sci. U. S. A.* **102,** 11296–301 (2005).
66. Baker, D. J. *et al.* BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. *Nat. Genet.* **36,** 744–9 (2004).
67. Ricke, R. M. & van Deursen, J. M. Aneuploidy in health, disease, and aging. *J. Cell Biol.* **201,** 11–21 (2013).
68. Janssen, A., Kops, G. J. P. L. & Medema, R. H. Elevating the frequency of chromosome mis-segregation as a strategy to kill tumour cells. *Proc. Natl. Acad. Sci. U. S. A.* **106,** 19108–13 (2009).
69. Tang, Y.-C., Williams, B. R., Siegel, J. J. & Amon, A. Identification of aneuploidy-selective antiproliferation compounds. *Cell* **144,** 499–512 (2011).
70. Wu, G. *et al.* Small molecule targeting the Hec1/Nek2 mitotic pathway suppresses tumour cell growth in culture and in animal. *Cancer Res.* **68,** 8393–9 (2008).
71. Kalitsis, P. *et al.* Increased chromosome instability but not cancer predisposition in haploinsufficient Bub3 mice. *Genes. Chromosomes Cancer* **44,** 29–36 (2005).
72. Li, M., Fang, X., Wei, Z., York, J. P. & Zhang, P. Loss of spindle assembly checkpoint-mediated inhibition of Cdc20 promotes tumourigenesis in mice. *J. Cell Biol.* **185,** 983–94 (2009).
73. Weaver, B. a a *et al.* Centromere-associated protein-E is essential for the mammalian mitotic checkpoint to prevent aneuploidy due to single chromosome loss. *J. Cell Biol.* **162,** 551–63 (2003).
74. Michel, L. S. *et al.* premature anaphase and chromosome instability in mammalian cells. 355–359 (2001).
75. Howman, E. V *et al.* Early disruption of centromeric chromatin organization in centromere protein A (Cenpa) null mice. *Proc. Natl. Acad. Sci. U. S. A.* **97,** 1148–53 (2000).
76. Hudson, D. F. *et al.* Centromere protein B null mice are mitotically and meiotically normal but have lower body and testis weights. *J. Cell Biol.* **141,** 309–19 (1998).
77. Kapoor, M. *et al.* The cenpB gene is not essential in mice. *Chromosoma* **107,** 570–6 (1998).
78. Perez-Castro, a V *et al.* Centromeric protein B null mice are viable with no apparent abnormalities. *Dev. Biol.* **201,** 135–43 (1998).
79. Kalitsis, P., Fowler, K. J., Earle, E., Hill, J. & Choo, K. H. Targeted disruption of mouse centromere protein C gene leads to mitotic disarray and early embryo death. *Proc. Natl. Acad. Sci. U. S. A.* **95,** 1136–41 (1998).
80. Mukherjee, M. *et al.* MMTV-Espl1 transgenic mice develop aneuploid, estrogen receptor alpha (ERα)-positive mammary adenocarcinomas. *Oncogene* 1–12 (2013). doi:10.1038/onc.2013.493
81. Wang, Z., Yu, R. & Melmed, S. Mice lacking pituitary tumour transforming gene show testicular and splenic hypoplasia, thymic hyperplasia, thrombocytopenia, aberrant cell cycle progression, and premature centromere division. *Mol. Endocrinol.* **15,** 1870–9 (2001).
82. Chesnokova, V., Kovacs, K., Castro, A.-V., Zonis, S. & Melmed, S. Pituitary hypoplasia in Pttg-/- mice is

protective for Rb+/- pituitary tumourigenesis. *Mol. Endocrinol.* **19,** 2371–9 (2005).
83. Abbud, R. a *et al.* Early multipotential pituitary focal hyperplasia in the alpha-subunit of glycoprotein hormone-driven pituitary tumour-transforming gene transgenic mice. *Mol. Endocrinol.* **19,** 1383–91 (2005).
84. Donangelo, I. *et al.* Pituitary tumour transforming gene overexpression facilitates pituitary tumour development. *Endocrinology* **147,** 4781–91 (2006).
85. Caldwell, C. M., Green, R. a & Kaplan, K. B. APC mutations lead to cytokinetic failures in vitro and tetraploid genotypes in Min mice. *J. Cell Biol.* **178,** 1109–20 (2007).
86. Oshima, M. *et al.* Loss of Apc heterozygosity and abnormal tissue building in nascent intestinal polyps in mice carrying a truncated Apc gene. *Proc. Natl. Acad. Sci. U. S. A.* **92,** 4482–6 (1995).
87. Rao, C. V *et al.* Colonic tumourigenesis in BubR1+/- Apc Min/- compound mutant mice is linked to premature separation of sister chromatids and enhanced genomic instability. *Proc. Natl. Acad. Sci. U. S. A.* **102,** 4365–4370 (2005).
88. Su, L. K. *et al.* Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. *Science (80-.).* **256,** 668–70 (1992).
89. Uren, A. G. *et al.* Survivin and the inner centromere protein INCENP show similar cell-cycle localization and gene knockout phenotype. *Curr. Biol.* **10,** 1319–28 (2000).
90. Zhang, D. *et al.* Cre-loxP-controlled periodic Aurora-A overexpression induces mitotic abnormalities and hyperplasia in mammary glands of mouse models. *Oncogene* **23,** 8720–30 (2004).
91. Zhang, D. *et al.* Aurora A overexpression induces cellular senescence in mammary gland hyperplastic tumours developed in p53-deficient mice. *Oncogene* **27,** 4305–14 (2008).
92. Lu, L.-Y. *et al.* Polo-like kinase 1 is essential for early embryonic development and tumour suppression. *Mol. Cell. Biol.* **28,** 6870–6 (2008).
93. Zhang, Y. *et al.* USP44 regulates centrosome positioning to prevent aneuploidy and suppress tumourigenesis. **122,** (2012).